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PTO/SB/21 (02-04)
Approved for use through 07/31/2006. OMB 0651-0031
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

TRANSMITTAL FORM

(to be used for all correspondence after initial filing)

Application Number	09/807,802
Filing Date	November 29, 2001
First Named Inventor	James M. Wilson
Art Unit	1635
Examiner Name	Brian A. Whiteman
Attorney Docket Number	GNV31AUSA

Certificate of Correction NOV 15 2004

Total Number of Pages in This Submission: 41

ENCLOSURES *(Check all that apply)*

- ☐ Fee Transmittal Form
- ☐ Fee Attached
- ☐ Amendment/Reply
- ☐ After Final
- ☐ Affidavits/declaration(s)
- ☐ Extension of Time Request
- ☐ Express Abandonment Request
- ☐ Information Disclosure Statement
- ☐ Certified Copy of Priority Document(s)
- ☐ Response to Missing Parts/ Incomplete Application
- ☐ Response to Missing Parts under 37 CFR 1.52 or 1.53
- ☐ Drawing(s)
- ☐ Licensing-related Papers
- ☐ Petition
- ☐ Petition to Convert to a Provisional Application
- ☐ Power of Attorney, Revocation Change of Correspondence Address
- ☐ Terminal Disclaimer
- ☐ Request for Refund
- ☐ CD, Number of CD(s) _____
- ☐ After Allowance communication to Technology Center (TC)
- ☐ Appeal Communication to Board of Appeals and Interferences
- ☐ Appeal Communication to TC **(Appeal Notice, Brief, Reply Brief)**
- ☐ Proprietary Information
- ☐ Status Letter
- ☑ Other Enclosure(s) (please Identify below):
  3 pp. Request for Certificate of correction under 35 USC Section 254
  3 pp. PTO/SB/44 form

Remarks

28pp. Copies of original spec. pages, examiners Amendment, examiners cited references, Response and IDS
6 pp. Copies of original patent pages marked in red
Customer No. 00270
Express Mail No. ER635161526US

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual name	HOWSON AND HOWSON Cathy A. Kodroff
Signature	Cathy A Kodroff
Date	November 9, 2004

CERTIFICATE OF TRANSMISSION/MAILING

I hereby certify that this correspondence is being facsimile transmitted to the USPTO or deposited with the United States Postal Service with sufficient postage as first class mail in an envelope addressed to: Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450 on the date shown below.

Typed or printed name			
Signature		Date	

GNV31AUSA

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Appln. No.	: 09/807,802	Confirmation No.: 5849
Applicant	: James M. Wilson et al	
Filed	: November 29, 2001	
Patent No.	: 6,759,237 B1	
Issued	: July 6, 2004	
TC/A.U.	: 1635	
Examiner	: Brian Whiteman	
Customer No.	: 00270	
Title	: ADENO-ASSOCIATED VIRUS SEROTYPE 1 NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME	

Commissioner for Patents
PO Box 1450
Alexandria, VA 22313-1450

Sir:

REQUEST FOR CERTIFICATE OF CORRECTION
UNDER 35 USC SECTION 254

The following errors were found in the above-identified patent:

(1) Front page, add US Patent No. "5,622,856" under (56) references cited

(2) Front page, under foreign patent documents, replace "WO03/983440" with -- WO03/093440 -- .

Express Mail No. ER635161526US

(3) Front page, under (57) Abstract, replace "An improved tet-repressible system is described in which the transgene is expressed under the control of a promoter which is activated upon binding of a fusion protein composed of a reverse tet repressor/activation domain to tet operator sequences located immediately upstream of the transgene promoter. The tet operator sequences are substantially free of interferon inducible response elements (ISRE). The reverse tet repressor is fused to an activation domain which lacks signals for protein clearance, thus extending expression of the fusion protein. Suitably, the tetO sequences are immediately upstream of a quiet promoter."

with

-- The nucleic acid sequences of adeno-associated virus (AAV) serotype 1 are provided, as are vectors and host cells containing these sequences and functional fragments thereof. Also provided are methods of delivering genes via AAV-1 derived vectors. -- .

(4) Col. 3, lines 15-18, replace "FIG. 2 illustrates the predicted secondary structure of AAV-1 ITR has been inserted after the word. The nucleotides in AAV-2 and AAV-6 are shown in italic and bold respectively"

with

-- FIG. 2 illustrates the predicted secondary structure of AAV-1 ITR nucleotides 1-143 of SEQ ID NO. 1. The nucleotides in AAV-2 and AAV-6 are shown in italic and bold respectively. -- .

(5) Col. 3, line 25-29, replace "FIG. 3B is a detailed illustration of a 71 bp homologous region among AAV-1 has been inserted after the word, AAV-2 has been inserted after the word and AAV-6 has been inserted after the word. Nucleotides that differ among these serotypes are indicated by arrows."

with

-- FIG. 3B is a detailed illustration of a 71 bp homologous region among AAV-1 nucleotides 438-531 of SEQ ID NO: 1, AAV-2 nucleotides 424-517 of SEQ ID NO: 18, and AAV-6 nucleotides 423-516 of SEQ ID NO: 19. Nucleotides that differ among these serotypes are indicated by arrows. -- .

(6) Col. 8, line 53, replace "vivo," with -- vitro, -- .

(7) Col. 9, line 47, replace "sued" with -- used -- .

(8) Col. 18, line 28, replace "($5x10^{1}$ genomes)" with -- ($5x10^{10}$ genomes) -- .

(9) Col. 91, line 48, replace "arc" with -- are -- .

It is requested that a Certificate of Correction be issued to correct the above errors in accordance with the enclosed Form PTO 1050, which is submitted herewith.

These errors are typographical in nature and make no substantive changes. All errors were made by the USPTO, no fee is due for correction of these errors.

Enclosed for each correction is a photocopy of the original specification pages, examiners amendment, examiners cited reference, response and an IDS with the relevant words or phrases highlighted in blue and the corresponding original patent with errors marked in red. These documents will support the USPTO errors.

The Director of the US Patent and Trademark Office is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees paid on the filing, or during prosecution of this application to Deposit Account No. 08-3040.

Respectfully submitted,

HOWSON AND HOWSON

Attorneys for the Applicants

BY Cathy A. Kodroff

Cathy A. Kodroff
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Spring House, PA 19477
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PTO/SB/17 (10-03)
Approved for use through 07/31/2006. OMB 0651-0032
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

FEE TRANSMITTAL for FY 2004

Effective 10/01/2003. Patent fees are subject to annual revision.

☐ Applicant claims small entity status. See 37 CFR 1.27

TOTAL AMOUNT OF PAYMENT ($) 180.00

Complete if Known	
Application Number	09/807,802
Filing Date	November 29, 2001
First Named Inventor	James M. Wilson
Examiner Name	Brian A. Whiteman
Art Unit	1635
Attorney Docket No.	GNV31AUSA/UPNK1774

METHOD OF PAYMENT *(check all that apply)*

☑ Check ☐ Credit card ☐ Money Order ☐ Other ☐ None

☑ Deposit Account:

Deposit Account Number: 08-3040

Deposit Account Name: HOWSON AND HOWSON

The Director is authorized to: *(check all that apply)*

☐ Charge fee(s) indicated below ☑ Credit any overpayments

☑ Charge any additional fee(s) or any underpayment of fee(s)

☐ Charge fee(s) indicated below, except for the filing fee to the above-identified deposit account.

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1001	770	2001	385	Utility filing fee	
1002	340	2002	170	Design filing fee	
1003	530	2003	265	Plant filing fee	
1004	770	2004	385	Reissue filing fee	
1005	160	2005	80	Provisional filing fee	

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES FOR UTILITY AND REISSUE

		Extra Claims		Fee from below	=	Fee Paid
Total Claims	9	-20** =	0	X 0	=	0
Independent Claims	1	-3** =	0	X 0	=	0
Multiple Dependent				0	=	0

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
1202	18	2202	9	Claims in excess of 20
1201	86	2201	43	Independent claims in excess of 3
1203	290	2203	145	Multiple dependent claim, if not paid
1204	86	2204	43	** Reissue independent claims over original patent
1205	18	2205	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($)

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
1051	130	2051	65	Surcharge - late filing fee or oath	
1052	50	2052	25	Surcharge - late provisional filing fee or cover sheet	
1053	130	1053	130	Non-English specification	
1812	2,520	1812	2,520	For filing a request for *ex parte* reexamination	
1804	920*	1804	920*	Requesting publication of SIR prior to Examiner action	
1805	1,840*	1805	1,840*	Requesting publication of SIR after Examiner action	
1251	110	2251	55	Extension for reply within first month	
1252	420	2252	210	Extension for reply within second month	
1253	950	2253	475	Extension for reply within third month	
1254	1,480	2254	740	Extension for reply within fourth month	
1255	2,010	2255	1,005	Extension for reply within fifth month	
1401	330	2401	165	Notice of Appeal	
1402	330	2402	165	Filing a brief in support of an appeal	
1403	290	2403	145	Request for oral hearing	
1451	1,510	1451	1,510	Petition to institute a public use proceeding	
1452	110	2452	55	Petition to revive - unavoidable	
1453	1,330	2453	665	Petition to revive - unintentional	
1501	1,330	2501	665	Utility issue fee (or reissue)	
1502	480	2502	240	Design issue fee	
1503	640	2503	320	Plant issue fee	
1460	130	1460	130	Petitions to the Commissioner	
1807	50	1807	50	Processing fee under 37 CFR 1.17(q)	
1806	180	1806	180	Submission of Information Disclosure Stmt	180.00
8021	40	8021	40	Recording each patent assignment per property (times number of properties)	
1809	770	2809	385	Filing a submission after final rejection (37 CFR 1.129(a))	
1810	770	2810	385	For each additional invention to be examined (37 CFR 1.129(b))	
1801	770	2801	385	Request for Continued Examination (RCE)	
1802	900	1802	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($) 180.00

(Complete *(if applicable)*)

SUBMITTED BY		Registration No. (Attorney/Agent)	33,980	Telephone	215-540-9200
Name (Print/Type)	Cathy A. Kodroff			Date	December 23, 2003
Signature	Cathy A. Kodroff				

WARNING: Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

This collection of information is required by 37 CFR 1.17 and 1.27. The information is required to obtain or retain a benefit by the public which is to file (and by th USPTO to process) an application. Confidentiality is governed by 35 U.S.C. 122 and 37 CFR 1.14. This collection is estimated to take 12 minutes to complet including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments c the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent ar Trademark Office, U.S. Department of Commerce, P.O. Box 1450, Alexandria, VA 22313-1450. DO NOT SEND FEES OR COMPLETED FORMS TO THIS A

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U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

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TRANSMITTAL FORM (to be used for all correspondence after initial filing)			
		Application Number	09/807,802
		Filing Date	November 29, 2001
		First Named Inventor	James M. Wilson
		Group Art Unit	1635
		Examiner Name	Brian A. Whiteman
Total Number of Pages in this Submission	14	Attorney Docket Number	GNV31AUSA/UPN-K1774

ENCLOSURES *(check all that apply)*

☒ Fee Transmittal Form	☐ Drawing(s)	☐ After Allowance Communication to Group
☒ Fee Attached	☐ Licensing-related Papers	☐ Appeal Communication to Board of Appeals and Interferences
☒ Amendment/Reply	☐ Petition	☐ Appeal Communication to Group (Appeal Notice, Brief, Reply Brief)
☐ After Final	☐ Petition to Convert to a Provisional Application	☐ Proprietary Information
☐ Affidavits/declaration(s)	☐ Power of Attorney, Revocation Change of Correspondence Address	☐ Status Letter
☐ Extension of Time Request	☐ Terminal Disclaimer	☒ Other Enclosure(s) (please identify below):
☐ Express Abandonment Request	☐ Request for Refund	12-References
☒ Information Disclosure Statement	☐ CD, Number of CD(s) ____	
☐ Certified Copy of Priority Document(s)	Remarks	
☐ Response to Missing Parts/ Incomplete Application		
☐ Response to Missing Parts under 37 CFR 1.52 or 1.53		

SIGNATURE OF APPLICANT, ATTORNEY, OR AGENT

Firm or Individual Name	HOWSON AND HOWSON Cathy A. Kodroff
Signature	Cathy A. Kodroff
Date	December 23, 2003

CERTIFICATE OF TRANSMISSION/MAILING

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PTO/SB/44 (04-04)
Approved for use through 04/30/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 6,759,237 B1
DATED : July 6, 2004
INVENTOR(S) : James M. Wilson and Weidong Xiao

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(1) Front page, add US Patent No. "5,622,856" under (56) references cited

(2) Front page, under foreign patent documents, replace "WO03/983440" with -- WO03/093440 -- .

(3) Front page, under (57) Abstract, replace "An improved tet-repressible system is described in which the transgene is expressed under the control of a promoter which is activated upon binding of a fusion protein composed of a reverse tet repressor/activation domain to tet operator sequences located immediately upstream of the transgene promoter. The tet operator sequences are substantially free of interferon inducible response elements (ISRE). The reverse tet repressor is fused to an activation domain which lacks signals for protein clearance, thus extending expression of the fusion protein. Suitably, the tetO sequences are immediately upstream of a quiet promoter."

with

-- The nucleic acid sequences of adeno-associated virus (AAV) serotype 1 are provided, as are vectors and host cells containing these sequences and functional fragments thereof. Also provided are methods of delivering genes via AAV-1 derived vectors. -- .

MAILING ADDRESS OF SENDER:

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Spring House Corporate Center
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Spring House, PA 19477

PATENT NO: 6,759,237

No. of additional copies

PTO/SB/44 (04-04)
Approved for use through 04/30/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 6,759,237 B1
DATED : July 6, 2004
INVENTOR(S) : James M. Wilson and Weidong Xiao

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with

-- FIG. 2 illustrates the predicted secondary structure of AAV-1 ITR nucleotides 1-143 of SEQ ID NO. 1. The nucleotides in AAV-2 and AAV-6 are shown in italic and bold respectively. -- .

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PATENT NO: 6,759,237

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PTO/SB/44 (04-04)
Approved for use through 04/30/2007. OMB 0651-0033
U.S. Patent and Trademark Office; U.S. DEPARTMENT OF COMMERCE

(Also Form PTO-1050)

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO : 6,759,237 B1
DATED : July 6, 2004
INVENTOR(S) : James M. Wilson and Weidong Xiao

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

(6) Col. 8, line 53, replace "vivo," with -- vitro, -- .

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(8) Col. 18, line 28, replace "($5x10^1$ genomes)" with -- ($5x10^{10}$ genomes) -- .

(9) Col. 91, line 48, replace "arc" with -- are -- .

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Spring House Corporate Center
Box 457
Spring House, PA 19477

PATENT NO: 6,759,237

No. of additional copies

Errors (1), (2), (3)

US006759237B1

(12) **United States Patent**
Wilson et al.

(10) **Patent No.: US 6,759,237 B1**
(45) **Date of Patent: Jul. 6, 2004**

(54) **ADENO-ASSOCIATED VIRUS SEROTYPE 1 NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME**

(75) Inventors: **James M. Wilson**, Gladwyne, PA (US); **Weidong Xiao**, Fort Washington, PA (US)

(73) Assignee: **The Trustees of the University of Pennsylvania**, Philadelphia, PA (US)

(*) Notice: Subject to any disclaimer, the term of this patent is extended or adjusted under 35 U.S.C. 154(b) by 0 days.

(21) Appl. No.: **09/807,802**

(22) PCT Filed: **Nov. 2, 1999**

(86) PCT No.: **PCT/US99/25694**

§ 371 (c)(1),
(2), (4) Date: **Nov. 29, 2001**

(87) PCT Pub. No.: **WO00/28061**

PCT Pub. Date: **May 18, 2000**

Related U.S. Application Data

(60) Provisional application No. 60/107,114, filed on Nov. 5, 1998.

(51) **Int. Cl.**[7] **C12N 15/00**; C12N 15/09; C12N 15/63; C12N 15/864; C12N 15/70; C12N 15/74

(52) **U.S. Cl.** **435/320.1**; 435/456; 435/325; 435/366; 435/367; 424/93.2

(58) **Field of Search** 435/320.1, 455, 435/456, 325, 20.1; 424/93.21, 93.2; 576/23.72

(56) **References Cited**

See attached Ref. Cited by Examiner 5,622,856

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6,156,303	A *	12/2000	Russell et al.	424/93.2
6,251,677	B1	6/2001	Wilson	435/457
6,258,595	B1	7/2001	Gao	435/320.1
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WO	WO 9613598	*	5/1996
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WO	WO98/10088	A1	3/1998
WO	WO98/11244	A2	3/1998
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WO	WO99/15677	A1	4/1999
WO	WO99/15685	A1	4/1999
WO	WO99/47691	A1	9/1999
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WO	WO01/23001	A2	4/2001
WO	WO01/23597	A2	4/2001
WO	WO01/40455	A2	6/2001
WO	WO01/83692	A2	11/2001
WO	WO03/092598	A2	11/2003
WO	WO03/983440	A2	11/2003

See attached S.IDS - 12-23-03

OTHER PUBLICATIONS

Rose et al., Microbiology, vol. 546, 1966, pp. 86–92.*
Anderson et al., Nature, vol. 392, pp 25–30, 1998.*
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R. Kotin et al, "Site–Specific integration by Adeno–Associated Virus", Proc. Natl. Acad. Sci. USA, 87:2211–2215 (Mar., 1990).
K. Fisher et al, "Recombinant Adeno–Associated Virus for Muscle Directed Gene Therapy", Nature Medicine, 3(3):306–312 (Mar., 1997).

(List continued on next page.)

Primary Examiner—Scott D. Priebe
Assistant Examiner—Brian Whiteman
(74) *Attorney, Agent, or Firm*—Howson and Howson

(57) **ABSTRACT**

An improved tet-repressible system is described in which the transgene is expressed under the control of a promoter which is activated upon binding of a fusion protein composed of a reverse tet repressor/activation domain to tet operator sequences located immediately upstream of the transgene promoter. The tet operator sequences are substantially free of interferon inducible response elements (ISRE). The reverse tet repressor is fused to an activation domain which lacks signals for protein clearance, thus extending expression of the fusion protein. Suitably, the tetO sequences are immediately upstream of a quiet promoter.

See attached Response dated 12-23-03 with label post-it

9 Claims, 13 Drawing Sheets

ERROR (1)

Notice of References Cited

Application/Control No.	Applicant(s)/Patent Under Reexamination	
09/807,802	WILSON ET AL.	
Examiner	Art Unit	Page 1 of 1
Brian Whiteman	1635	

U.S. PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Name	Classification
	A	US-5,622,856	04-1997	Natsoulis	435/325
	B	US-			
	C	US-			
	D	US-			
	E	US-			
	F	US-			
	G	US-			
	H	US-			
	I	US-			
	J	US-			
	K	US-			
	L	US-			
	M	US-			

FOREIGN PATENT DOCUMENTS

*		Document Number Country Code-Number-Kind Code	Date MM-YYYY	Country	Name	Classification
	N					
	O					
	P					
	Q					
	R					
	S					
	T					

NON-PATENT DOCUMENTS

*		Include as applicable: Author, Title Date, Publisher, Edition or Volume, Pertinent Pages)
	U	Rutledge et al., Virol 1998; 72:309-19
	V	
	W	
	X	

*A copy of this reference is not being furnished with this Office action. (See MPEP § 707.05(a).)
Dates in MM-YYYY format are publication dates. Classifications may be US or foreign.

GNV31AUSA/UPN-K1774

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of James M. Wilson	) Group Art Unit: 1635)) Examiner: Brian A. Whiteman
Appln. No. 09/807,802	) Confirmation No. 5849
Filed: November 29, 2001	)
For: ADENO-ASSOCIATED VIRUS SEROTYPE I NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME	) December 23, 2003

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

SUPPLEMENTAL INFORMATION DISCLOSURE STATEMENT

Sir:

Applicant submits to the Examiner the attached Form PTO/SB/08A/B document listing and this paper pursuant to 37 CFR § 1.56 and § 1.97-1.98. Form PTO/SB/08A/B is attached and a copy of the documents are enclosed herewith. This Information Disclosure Statement is submitted more than three months from the filing date of this application and after the receipt of a first Office Action on the merits. Therefore, a fee of $180.00 is due.

The Director is hereby authorized to charge any deficiency in any fees due with the filing of this paper or credit any overpayment in any fees to our Deposit Account Number 08-3040.

EXPRESS MAIL NO. EU531571956US CUSTOMER NO. 00270

The Examiner is respectfully requested to consider the enclosed document identified in this paper and in the attached Form PTO/SB/08A/B during the course of examination of this application.

Respectfully submitted,

HOWSON AND HOWSON
Attorneys for Applicant

By _____

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PTO/SB/08B (04-03)
Approved for use through 4/30/2003. OMB 0651-0031
U.S. Patent and Trademark Office;
U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 1 of 2

Complete if Known	
Application Number	09/807,802
Filing Date	April 17, 2001
First Named Inventor	James M. Wilson
Group Art Unit	1635
Examiner Name	Brian A. Whiteman
Attorney Docket Number	GNV31AUSA/UPN-K1774

U.S. PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Document Number Number-Kind Code[2](if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear
	CA	US-6,387,368 B1	05-14-2002	Wilson et al	
	CB	US-6,274,354 B1	08-14-2001	Wilson et al	
	CC	US-6,482,634 B1	11-19-2002	Wilson et al	
	CD	US-6,475,769 B1	11-05-2002	Wilson et al	
	CE	US-6,365,394 B1	04-02-2002	Gao et al	
	CF	US-6,399,385 B1	06-04-2002	Croyle et al	

FOREIGN PATENT DOCUMENTS

Examiner Initials*	Cite No.[1]	Foreign Patent Document Country Code[3]- Number[4]-Kind Code[5](if known)	Publication Date MM-DD-YYYY	Name of Patentee or Applicant of Cited Document	Pages, Columns, Lines, Where Relevant Passages or Relevant Figures Appear	T[6]
	CG	PCT WO03/093440 A2	11-13-2003	University of Florida		
	CH	PCT WO03/092598 A2	11-13-2003	University of Florida		

Examiner Signature		Date Considered	

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] See Kinds Codes of USPTO Patent Documents at www.uspto.gov or MPEP 901.04. [3] Enter Office that issued the document, by the two-letter code (WIPO Standard ST.3). [4] For Japanese patent documents, the indication of the year of the reign of the Emperor must precede the serial number of the patent document. [5] Kind of document by the appropriate symbols as indicated on the document under WIPO Standard ST. 16 if possible. [6] Applicant is to place a check mark here if English language Translation is attached.

This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 USC 122 and 37 CFR 1.14. this collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETE FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, Washington, DC 20231.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

PTO/SB/08B (08-00)

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through 4/30/2003.
OMB 0651-0031
U.S. Patent and Trademark Office;
U.S. DEPARTMENT OF COMMERCE

Substitute for form 1449/PTO

INFORMATION DISCLOSURE STATEMENT BY APPLICANT

(Use as many sheets as necessary)

Sheet 2 of 2

Complete if Known	
Application Number	09/807,802
Filing Date	April 17, 2001
First Named Inventor	James M. Wilson
Group Art Unit	1635
Examiner Name	Brian A. Whiteman
Attorney Docket Number	GNV31AUSA/UPN-K1774

OTHER PRIOR ART-NONPATENT LITERATURE DOCUMENTS

Examiner Initials*	Cite No.[1]	Include the name of the author (in CAPITAL LETTERS), title of the article (when appropriate), title of the item, (book, magazine, journal, serial, symposium, catalog, etc.), date, page(s), columeousse number(s), publisher, city and/or country where published	T[2]
	CI	HAUCK ET AL, Generation and Characterization of Chimeric Recombinant AAV Vectors, Molecular Therapy, Vol. 7, No. 3, pp. 419-425, (March 2003)	
	CJ	RABINOWITZ ET AL, Cross-Packaging of a Single Adeno-Associated Virus (AAV) Type 2 Vector Genome into Multiple AAV Serotypes Enables Transduction with Broad Specificity, Journal of Virology, Vol. 76, No. 2, pp. 791-801, (January 2002)	
	CK	HAUCK ET AL, Characterization of Tissue Tropism Determinants of Adeno-Associated Virus Type 1, Journal of Virology, Vol. 77, No. 4, pp. 2768-2774, (February 2003)	
	CL	ARRUDA ET AL, Safety and Efficacy of Factor IX Gene Transfer to Skeletal Muscle in Murine and Canine Hemophilia B Models by Adeno-Associated Viral Vector Serotype 1, Blood First Edition Paper, (September 11, 2003)	

Examiner Signature		Date Considered	

* EXAMINER: Initial if reference considered, whether or not citation is in conformance with MPEP 609. Draw line through citation if not in conformance and not considered. Include copy of this form with next communication to applicant. [1] Applicant's unique citation designation number (optional). [2] Applicant is to place a check mark here if English language Translation is attached. This collection of information is required by 37 CFR 1.97 and 1.98. The information is required to obtain or retain a benefit by the public which is to file (and by the USPTO to process) an application. Confidentiality is governed by 35 USC 122 and 37 CFR 1.14. this collection is estimated to take 2 hours to complete, including gathering, preparing, and submitting the completed application form to the USPTO. Time will vary depending upon the individual case. Any comments on the amount of time you require to complete this form and/or suggestions for reducing this burden, should be sent to the Chief Information Officer, U.S. Patent and Trademark Office, U.S. Department of Commerce, Washington, DC 20231. DO NOT SEND FEES OR COMPLETE FORMS TO THIS ADDRESS. **SEND TO: Commissioner for Patents, Washington, DC 20231.**

If you need assistance in completing the form, call 1-800-PTO-9199 (1-800-786-9199) and select option 2.

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of	) Group Art Unit: 1635
James M. Wilson et al	) Examiner: B. Whiteman
Appln. No. 09/807,802	) Confirmation No. 5849
Filed: November 29, 2001	)
For: ADENO-ASSOCIATED VIRUS SEROTYPE I NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME	) December 23, 2003

Mail Stop Non-Fee Amendment
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

RESPONSE PURSUANT TO 37 CFR 1.111

Sirs:

This is in timely response to the Office Action dated September 25, 2003. Kindly amend the application as follows.

In the Specification:

Add the attached abstract of the disclosure, provided herewith on a separate sheet.

Other specification amendments are provided on a separate sheet.

In the Claims:

Claims 9, 10, 11, 14, 17, 18, 19, 20, 29, 30 and 37-40 are cancelled, without prejudice. Kindly add new claims 41 – 45 as follows.

Amendments to the Specification:

Kindly replace the paragraph at page 12, lines 15 – 26, with the following:

- -

Alternatively, the cis plasmid and, optionally, the trans plasmid, may be transfected into a packaging cell line which provides the remaining helper functions necessary for production of a rAAV containing the desired AAV-1 sequences of the invention. An example of a suitable packaging cell line, wherein an AAV=2 capsid is desired, is B-50, which stably expresses AAV-2 rep and cap genes under the control of a homologous P5 promoter. This cell line is characterized by integration into the cellular chromosome of multiple copies (at least 5 copies) of P5-rep-cap gene in a concatomer form. This B-50 cell line was deposited with the ~~American Type Culture Collection~~ AMERICAN TYPE CULTURE COLLECTION depositary, 10801 University Boulevard, Manassas, Virginia 20110-2209, on September 18, 1997 under Accession No. CRL-12401 pursuant to the provisions of the Budapest Treaty. However, the present invention is not limited as to the selection of the packaging cell line. - -

Replace the paragraph at page 14, lines 16 – 26 with the following:

- - Alternatively, host cells of the invention may be stably transfected with a rAAV expression cassette of the invention, and with copies of AAV-1 rep and cap genes. Suitable parental cell lines include mammalian cell lines and it may be desirable to select host cells from among non-simian mammalian cells. Examples of suitable parental cell lines include, without limitation, HeLa [ATCC® depositary, Accession No. CCL 2], A549 [ATCC® depositary, Accession No. CCL 185], KB [CCL 17], Detroit [e.g., Detroil 510, CCL 72] and WI-38 [CCL 75] cells. These cell

lines are all available from the ~~American Type Culture Collection~~ AMERICAN TYPE CULTURE COLLECTION depositary, 10801 University Boulevard, Manassas, Virginia 20110-220910801 University Boulevard, Manassas, Virginia 20110-2209 USA. Other suitable parent cell lines may be obtained from other sources and may be sued to construct stable cell lines containing the P5 and/or AAV rep and cap sequences of the invention. - -

Amendments to the Claims:

Claims 1 and 2. Currently Cancelled.

Claim 3. Previously canceled.

Claim 4. Currently Cancelled.

Claims 5 – 8. Previously Cancelled.

Claims 9 – 11. Currently Cancelled.

Claims 12 and 13. Previously Cancelled.

Claim 14. Currently Cancelled.

Claim 15. Previously Cancelled.

Claims 16 – 20. Currently Cancelled.

Claims 21 and 22. Previously Cancelled.

Claims 23 – 25. Currently Cancelled.

Claim 26. Previously cancelled.

Claims 27 – 32. Currently cancelled.

33 (Previously Presented). A recombinant virus having an AAV-1 capsid comprising an AAV-1 protein selected from among AAV-1 vp1 having the amino acid sequence of SEQ ID NO:13; AAV-1 vp2 having the amino acid sequence of SEQ ID NO:15 and AAV-1 vp3 having the amino acid sequence of SEQ ID NO:17 and a heterologous molecule which comprises an AAV 5' inverted terminal repeat sequence (ITR), a transgene, and an AAV 3' ITR.

34 (Previously Presented). The recombinant virus according to claim 33, wherein the 5' ITR and 3' ITR are of AAV serotype 2.

35 (Previously Presented). The recombinant virus according to claim 33 further comprising a regulatable promoter which directs expression of the transgene.

36 (Previously Presented). A recombinant host cell transformed with the recombinant virus of claim 33.

Claims 37 – 40. Currently Cancelled.

41 (New). The recombinant virus according to claim 33, wherein said virus comprises an AAV-1 capsid comprising the AAV-1 vp1 having the amino acid sequence of SEQ ID NO:13.

42 (New). The recombinant virus according to claim 33, wherein said virus comprises an AAV-1 capsid comprising the AAV-1 vp2 having the amino acid sequence of SEQ ID NO:15.

43 (New). The recombinant virus according to claim 33, wherein said virus comprises an AAV-1 capsid comprising the AAV-1 vp3 having the amino acid sequence of SEQ ID NO:17.

44 (New). The recombinant virus according to claim 33, wherein the 5' ITR and 3' ITR are of AAV serotype 1.

45 (New). A composition comprising a carrier and a recombinant virus according to claim 33.

REMARKS

Claims 33 – 45 are pending.

Claims 1, 2, 4, 21, 22-25, 27-29, 32 are being cancelled, without prejudice, in order to reduce the issues in this application and expedite allowance thereof. Cancellation of these claims renders the rejections and objections thereto moot.

Claims 9-11, 14, 17-20, 29, 30, and 37-40 are cancelled as being drawn to non-elected subject matter.

Claims 41 - 45 are new. Claims 41 – 43 are supported on page 8, lines 22-23; page 11, lines 7-11. Claim 44 is supported on page 11, lines 7-11 and page 13, lines 13-14. Claim 45 is supported page 16, lines 9-12.

These amendments are further supported throughout the specification.

No new matter is added by this amendment.

I. Specification

An abstract on a separate sheet is being provided herewith. This abstract is identical to that provided on the cover sheet of the published WO.

The Examiner has noted the improper usage of certain trademarks in the application. Applicants have performed a search and have discovered that ATCC is a trademark of the American Type Cell Culture. The specification has been corrected in the appropriate locations. However, Applicants have been unable to discover any claim that the phrase "American Type Cell Culture" is itself a trademark. In fact, the "American Type Cell Culture" is identified on the organization's web cite as the owner of a variety of trademarks and the name under which the organization does business.

No new matter is added by this amendment.

II. Election

Applicants note that the restriction requirement has been made final and have cancelled withdrawn claims 9 - 11, 14, 17-20, 29, 30 and 37-40, without prejudice.

(12) PCT

WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 : C12N 15/86, 15/35, 5/10, A61K 48/00 — A3

(11) International Publication Number: WO 00/28061

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(21) International Application Number: PCT/US99/25694

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(30) Priority Data:
60/107,114 5 November 1998 (05.11.98) US

(71) Applicant *(for all designated States except US)*: THE TRUSTEES OF THE UNIVERSITY OF PENNSYLVANIA [US/US]; Suite 300, 3700 Market Street, Philadelphia, PA 19104–3147 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: WILSON, James, M. [US/US]; 1350 N. Avignon Drive, Gladwyne, PA 19035 (US). XIAO, Weidong [CN/US]; Apartment P4, 155 Washington Lane, Jenkintown, PA 19046 (US).

(74) Agents: KODROFF, Cathy, A. et al.; Howson & Howson, Spring House Corporate Center, P.O. Box 457, Spring House, PA 19477 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
With international search report.

(88) Date of publication of the international search report:
3 August 2000 (03.08.00)

(54) Title: ADENO–ASSOCIATED VIRUS SEROTYPE 1 NUCLEIC ACID SEQUENCES, VECTORS AND HOST CELLS CONTAINING SAME

(57) Abstract

The nucleic acid sequences of adeno–associated virus (AAV) serotype 1 are provided, as are vectors and host cells containing these sequences and functional fragments thereof. Also provided are methods of delivering genes via AAV–1 derived vectors.

(ERROR 3)

Express Mail No. ET033648243US

III. Allowable Subject Matter.

The examiner has indicated that Claims 33-36 are in condition for allowance, because they are free of the prior art of record. A number of claims dependent therefrom have been added.

Applicants believe that the claims are in good condition to pass to issue. The undersigned attorney requests that the examiner telephone if doing so will expedite processing of this application.

No fees are believed to be associated with the filing of this response. However, the Director of the US Patent and Trademark Office is hereby authorized to charge any fee associated with the filing of this paper to deposit account 08-3040.

Respectfully submitted,
HOWSON AND HOWSON
Attorneys for the Applicant

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ERRORS (4) and (5)

Other aspects and advantages of the invention will be readily apparent to one of skill in the art from the detailed description of the invention.

BRIEF DESCRIPTION OF THE DRAWING

FIGS. 1A–1F illustrate the alignment of nucleotides of AAV-1 [SEQ ID NO: 1], AAV-2 [SEQ ID NO: 18] and AAV-6 [SEQ ID NO: 19]. The alignment was done with MacVector 6.0. The full sequences of AAV-1 are shown in the top line. Nucleotides in AAV-2 and AAV-6 identical to AAV-1 are symbolized by "." and gaps by "-". Some of the conserved features among AAVs are marked in this figure. Note the 3' ITRs of AAV-1 and AAV-6 are shown in different orientations.

FIG. 2 illustrates the predicted secondary structure of AAV-1 ITR has been inserted after the word. The nucleotides in AAV-2 and AAV-6 are shown in italic and bold respectively.

See attached Examiner's Amendment

FIG. 3A illustrates a hypothesis of how AAV-6 arose from the homologous recombination between AAV-1 and AAV-2. The major elements of AAV-1 are indicated in the graph. A region that is shared between AAV-1, AAV-2 and AAV-6 is shown in box with waved lines.

FIG. 3B is a detailed illustration of a 71 bp homologous region among AAV-1 has been inserted after the word, AAV-2 has been inserted after the word and AAV-6 has been inserted after the word. Nucleotides that differ among these serotypes are indicated by arrows.

FIG. 4A is a bar chart illustrating expression levels of human alpha 1 anti-trypsin (α1AT) in serum following delivery of hAAT via recombinant AAV-1 and recombinant AAV-2 viruses.

FIG. 4B is a bar chart illustrating expression levels of erythropoietin (epo) in serum following delivery of the epo gene via recombinant AAV-1 and recombinant AAV-2 viruses.

FIG. 5A is a bar chart illustrating expression levels of α1AT in liver following delivery of α1AT as described in Example 7.

FIG. 5B is a bar chart demonstrating expression levels of epo in liver following delivery of epo as described in Example 7.

FIG. 5C is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-1 following delivery of α1AT or epo to liver as described in Example 7.

FIG. 5D is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-2 following delivery of α1AT or epo to liver as described in Example 7.

FIG. 6A is a bar chart illustrating expression levels of α1AT in muscle following delivery of α1AT as described in Example 7.

FIG. 6B is a bar chart demonstrating expression levels of epo in muscle following delivery of epo as described in Example 7.

FIG. 6C is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-1 following delivery of α1AT or epo to muscle as described in Example 7.

FIG. 6D is a bar chart demonstrating neutralizing antibodies (NAB) directed to AAV-2 following delivery of α1AT or epo to muscle as described in Example 7.

DETAILED DESCRIPTION OF THE INVENTION

The present invention provides novel nucleic acid sequences for an adeno—associated virus of serotype 1 (AAV-1). Also provided are fragments of these AAV-1 sequences. Among particularly desirable AAV-1 fragments are the inverted terminal repeat sequences (ITRs), rep and cap. Each of these fragments may be readily utilized, e.g., as a cassette, in a variety of vector systems and host cells. Such fragments may be used alone, in combination with other AAV-1 sequences or fragments, or in combination with elements from other AAV or non-AAV viral sequences. In one particularly desirable embodiment, a cassette may contain the AAV-1 ITRs of the invention flanking a selected transgene. In another desirable embodiment, a cassette may contain the AAV-1 rep and/or cap proteins, e.g., for use in producing recombinant (rAAV) virus.

Thus, the AAV-1 sequences and fragments thereof are useful in production of rAAV, and are also useful as antisense delivery vectors, gene therapy vectors, or vaccine vectors. The invention further provides nucleic acid molecules, gene delivery vectors, and host cells which contain the AAV-1 sequences of the invention. Also provided a novel methods of gene delivery using AAV vectors.

As described herein, the vectors of the invention containing the AAV-1 capsid proteins of the invention are particularly well suited for use in applications in which the neutralizing antibodies diminish the effectiveness of other AAV serotype based vectors, as well as other viral vectors. The rAAV vectors of the invention are particularly advantageous in rAAV readministration and repeat gene therapy.

These and other embodiments and advantages of the invention are described in more detail below. As used throughout this specification and the claims, the term "comprising" is inclusive of other components, elements, integers, steps and the like.

I. AAV-1 Nucleic Acid and Protein Sequences

The AAV-1 nucleic acid sequences of the invention include the DNA sequences of SEQ ID NO: 1 (FIGS. 1A–1F), which consists of 4718 nucleotides. The AAV-1 nucleic acid sequences of the invention further encompass the strand which is complementary to SEQ ID NO: 1, as well as the RNA and cDNA sequences corresponding to SEQ ID NO: 1 and its complementary strand. Also included in the nucleic acid sequences of the invention are natural variants and engineered modifications of SEQ ID NO: 1 and its complementary strand. Such modifications include, for example, labels which are known in the art, methylation, and substitution of one or more of the naturally occurring nucleotides with an analog.

Further included in this invention are nucleic acid sequences which are greater than 85%, preferably at least about 90%, more preferably at least about 95%, and most preferably at least about 98–99% identical or homologous to SEQ ID NO: 1. The term "percent sequence identity" or "identical" in the context of nucleic acid sequences refers to the residues in the two sequences which are the same when aligned for maximum correspondence. The length of sequence identity comparison may be over the full-length sequence, or a fragment at least about nine nucleotides, usually at least about 20–24 nucleotides, at least about 28–32 nucleotides, and preferably at least about 36 or more nucleotides. There are a number of different algorithms known in the art which can be used to measure nucleotide sequence identity. For instance, polynucleotide sequences can be compared using Fasta, a program in GCG Version 6.1. Fasta provides alignments and percent sequence identity of the regions of the best overlap between the query and search sequences (Pearson, 1990, herein incorporated by

ERRORS (4) and (5)

EXAMINER'S AMENDMENT

An examiner's amendment to the record appears below. Should the changes and/or additions be unacceptable to applicant, an amendment may be filed as provided by 37 CFR 1.312. To ensure consideration of such an amendment, it MUST be submitted no later than the payment of the issue fee.

Authorization for this examiner's amendment was given in a telephone interview with Cathy Kodroff on 2/10/04.

The amendment to the specification filed on 4/17/01 was inserted before line 3 of page 1 rather than before line 6 of page 1 as directed.

The term "a" before the word recombinant virus on line 1 of claim 45 has been replaced with the term -- the --.

The term "Figs. 1A-1C" on page 4, line 6 has been replaced with -- Figs. 1A-1F --.

The term "Figs. 1A-1C" on page 6, line 13 has been replaced with -- Figs. 1A-1F --.

The term "Figs. 1A-1C" on page 20, line 13 has been replaced with -- Figs. 1A-1F --.

The term "Figs. 1A-1C" on page 21, line 20 has been replaced with -- Figs. 1A-1F --.

ERRORS (4) and (5)

The term "Figs. 1A-1C" on page 22, line 15 has been replaced with -- Figs. 1A-1F --.

The term "Figs. 1A-1C" on page 23, line 15 has been replaced with -- Figs. 1A-1F --.

The term -- (nucleotides 1-143 of SEQ ID NO: 1) -- has been inserted after the word AAV-1 ITR on line 12 of page 4.

The term -- (nucleotides 438-531 of SEQ ID NO: 1) -- has been inserted after the word AAV-1 on line 18 of page 4.

The term -- (nucleotides 424-517 of SEQ ID NO: 18) -- has been inserted after the word AAV-2 on line 19 of page 4.

The term -- (nucleotides 423-516 of SEQ ID NO: 19) -- has been inserted after the word AAV-6 on line 19 of page 4.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Brian Whiteman whose telephone number is (571) 272-0764. The examiner can normally be reached on Monday through Friday from 7:00 to 4:00 (Eastern Standard Time), with alternating Fridays off.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, John LeGuyader, SPE - Art Unit 1635, can be reached at (571) 272-0760.

Papers related to this application may be submitted to Group 1600 by facsimile transmission. Papers should be faxed to Group 1600 via the PTO Fax Center located in Crystal Mall 1. The faxing of such papers must conform with the notice published in the Official Gazette, 1096 OG 30 (November 15, 1989). The CM1 Fax Center number is (703) 872-9306.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Brian Whiteman
Patent Examiner, Group 1635

Scott D. Priebe

SCOTT D. PRIEBE, PH.D
PRIMARY EXAMINER

ERROR (6)

tion may also contain such an intron, desirably located between the promoter/enhancer sequence and the transgene. Selection of these and other common vector elements are conventional [see, e.g., Sambrook et al, "Molecular Cloning. A Laboratory Manual", 2d edit., Cold Spring Harbor Laboratory, New York (1989) and references cited therein] and many such sequences are available from commercial and industrial sources as well as from Genebank.

The selection of the transgene is not a limitation of the present invention. Suitable transgenes may be readily selected from among desirable reporter genes, therapeutic genes, and optionally, genes encoding immunogenic polypeptides. Examples of suitable reporter genes include β-galactosidase (β-gal), an alkaline phosphatase gene, and green fluorescent protein (GFP). Examples of therapeutic genes include, cytokines, growth factors, hormones, and differentiation factors, among others. The transgene may be readily selected by one of skill in the art. See, e.g., WO 98/09657, which identifies other suitable transgenes.

Suitably, the vectors of the invention contain, at a minimum, cassettes which consist of fragments of the AAV-1 sequences and proteins. In one embodiment, a vector of the invention comprises a selected transgene, which is flanked by a 5' ITR and a 3' ITR, at least one of which is an AAV-1 ITR of the invention. Suitably, vectors of the invention may contain a AAV-1 P5 promoter of the invention. In yet another embodiment, a plasmid or vector of the invention contains AAV-1 rep sequences. In still another embodiment, a plasmid or vector of the invention contains at least one of the AAV-1 cap proteins of the invention. Most suitably, these AAV-1-derived vectors are assembled into viral vectors, as described herein.

A. AAV Viral Vectors

In one aspect, the present invention provides a recombinant AAV-1 viral vector produced using the AAV-1 capsid proteins of the invention. The packaged rAAV-1 virions of the invention may contain, in addition to a selected minigene, other AAV-1 sequences, or may contain sequences from other AAV serotypes.

Methods of generating rAAV virions are well known and the selection of a suitable method is not a limitation on the present invention. See, e.g., K. Fisher et al, *J. Virol.*, 70:520–532 (1993) and U.S. Pat. No. 5,478,745. In one suitable method, a selected host cell is provided with the AAV sequence encoding a rep protein, the gene encoding the AAV cap protein and with the sequences for packaging and subsequent delivery. Desirably, the method utilizes the sequences encoding the AAV-1 rep and/or cap proteins of the invention.

In one embodiment, the rep/cap genes and the sequences for delivery are supplied by co-transfection of vectors carrying these genes and sequences. In one currently preferred embodiment, a cis (vector) plasmid, a trans plasmid containing the rep and cap genes, and a plasmid containing the adenovirus helper genes are co-transfected into a suitable cell line, e.g., 293. Alternatively, one or more of these functions may be provided in trans via separate vectors, or may be found in a suitably engineered packaging cell line.

An exemplary cis plasmid will contain, in 5' to 3' order, AAV 5' ITR, the selected transgene, and AAV 3' ITR. In one desirable embodiment, at least one of the AAV ITRs is a 143 nt AAV-1 ITR. However, other AAV serotype ITRs may be readily selected. Suitably, the full-length ITRs are utilized. However, one of skill in the art can readily prepare modified AAV ITRs using conventional techniques. Similarly, methods for construction of such plasmids is well known to those of skill in the art.

A trans plasmid for use in the production of the rAAV-1 virion particle may be prepared according to known techniques. In one desired embodiment, this plasmid contains the rep and cap proteins of AAV-1, or functional fragments thereof. Alternatively, the rep sequences may be from another selected AAV serotype.

The cis and trans plasmid may then be co-transfected with a wild-type helper virus (e.g., Ad2, Ad5, or a herpesvirus), or more desirably, a replication—defective adenovirus, into a selected host cell. Alternatively, the cis and trans plasmid may be co-transfected into a selected host cell together with a transfected plasmid which provides the necessary helper functions. Selection of a suitable host cell is well within the skill of those in the art and include such mammalian cells as 293 cells, HeLa cells, among others.

Alternatively, the cis plasmid and, optionally, the trans plasmid, may be transfected into a packaging cell line which provides the remaining helper functions necessary for production of a rAAV containing the desired AAV-1 sequences of the invention. An example of a suitable packaging cell line, wherein an AAV-2 capsid is desired, is B-50, which stably expresses AAV-2 rep and cap genes under the control of a homologous P5 promoter. This cell line is characterized by integration into the cellular chromosome of multiple copies (at least 5 copies) of P5-rep-cap gene in a concatomer form. This B-50 cell line was deposited with the AMERICAN TYPE CULTURE COLLECTION depositary, 10801 University Boulevard, Manassas, Va. 20110-2209, on Sep. 18, 1997 under Accession No. CRL-12401 pursuant to the provisions of the Budapest Treaty. However, the present invention is not limited as to the selection of the packaging cell line.

Exemplary transducing vectors based on AAV-1 capsid proteins have been tested both in vivo and in vitro, as described in more detail in Example 4. In these studies, it was demonstrated that recombinant AAV vector with an AAV-1 virion can transduce both mouse liver and muscle. These, and other AAV-1 based gene therapy vectors which may be generated by one of skill in the art are beneficial for gene delivery to selected host cells and gene therapy patients since the neutralization antibodies of AAV-1 present in much of the human population exhibit different patterns from other AAV serotypes and therefore do not neutralize the AAV-1 virions. One of skill in the art may readily prepare other rAAV viral vectors containing the AAV-1 capsid proteins provided herein using a variety of techniques known to those of skill in the art. One may similarly prepare still other rAAV viral vectors containing AAV-1 sequence and AAV capsids of another serotype.

B. Other Viral Vectors

One of skill in the art will readily understand that the AAV-1 sequences of the invention can be readily adapted for use in these and other viral vector systems for in vivo, ex vivo or in vivo gene delivery. Particularly well suited for use in such viral vector systems are the AAV-1 ITR sequences, the AAV-1 rep, the AAV-1 cap, and the AAV-1 P5 promoter sequences.

For example, in one desirable embodiment, the AAV-1 ITR sequences of the invention may be used in an expression cassette which includes AAV-1 5' ITR, a non-AAV DNA sequences of interest (e.g., a minigene), and 3' ITR and which lacks functional rep/cap. Such a cassette containing an AAV-1 ITR may be located on a plasmid for subsequent transfection into a desired host cell, such as the cis plasmid described above. This expression cassette may further be provided with an AAV capsid of a selected serotype to permit infection of a cell or stably transfected into a desired

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gene therapy vectors which may be generated by one of skill in the art are beneficial for gene delivery to selected host cells and gene therapy patients since the neutralization antibodies of AAV-1 present in much of the human population exhibit different patterns from other AAV serotypes and therefore do not neutralize the AAV-1 virions. One of skill in the art may readily prepare other rAAV viral vectors containing the AAV-1 capsid proteins provided herein using a variety of techniques known to those of skill in the art. One may similarly prepare still other rAAV viral vectors containing AAV-1 sequence and AAV capsids of another serotype.

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Another aspect of the present invention is the novel AAV-1 P5 promoter sequences which are located in the region spanning nt 236 - 299 of SEQ ID NO: 1. This promoter is useful in a variety of viral vectors for driving expression of a desired transgene.

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host cell for packaging of rAAV virions. Such an expression cassette may be readily adapted for use in other viral systems, including adenovirus systems and lentivirus systems. Methods of producing Ad/AAV vectors are well known to those of skill in the art. One desirable method is described in PCT/US95/14018. However, the present invention is not limited to any particular method.

Another aspect of the present invention is the novel AAV-1 P5 promoter sequences which are located in the region spanning nt 236–299 of SEQ ID NO: 1. This promoter is useful in a variety of viral vectors for driving expression of a desired transgene.

Similarly, one of skill in the art can readily select other fragments of the AAV-1 genome of the invention for use in a variety of vector systems. Such vectors systems may include, e.g., lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adenoviral systems, among others. Selection of these vector systems is not a limitation of the present invention.

C. Host Cells and Packaging Cell Lines

In yet another aspect, the present invention provides host cells which may be transiently transfected with AAV-1 nucleic acid sequences of the invention to permit expression of a desired transgene or production of a rAAV particle. For example, a selected host cell may be transfected with the AAV-1 P5 promoter sequences and/or the AAV-1 5' ITR sequences using conventional techniques. Providing AAV helper functions to the transfected cell lines of the invention results in packaging of the rAAV as infectious rAAV particles. Such cell lines may be produced in accordance with known techniques [see, e.g, U.S. Pat. No. 5,658,785], making use of the AAV-1 sequences of the invention.

Alternatively, host cells of the invention may be stably transfected with a rAAV expression cassette of the invention, and with copies of AAV-1 rep and cap genes. Suitable parental cell lines include mammalian cell lines and it may be desirable to select host cells from among non-simian mammalian cells. Examples of suitable parental cell lines include, without limitation, HeLa [ATCC® depositary, Accession No. CCL 2], A549 [ATCC® depositary, Accession No. CCL 185], KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells. These cell lines are all available from the AMERICAN TYPE CULTURE COLLECTION depositary, 10801 University Boulevard, Manassas, Va. 20110-220910801 University Boulevard, Manassas, Va. 20110-2209 USA. Other suitable parent cell lines may be obtained from other sources and may be sued to construct stable cell lines containing the P5 and/or AAV rep and cap sequences of the invention.

Recombinant vectors generated as described above are useful for delivery of the DNA of interest to cells.

III Methods of Delivering Genes via AAV-1 Derived Vectors

In another aspect, the present invention provides a method for delivery of a transgene to a host which involves transfecting or infecting a selected host cell with a recombinant viral vector generated with the AAV-1 sequences (or functional fragments thereof) of the invention. Methods for delivery are well known to those of skill in the art and are not a limitation of the present invention.

In one desirable embodiment, the invention provides a method for AAV—mediated delivery of a transgene to a host. This method involves transfecting or infecting a selected host cell with a recombinant viral vector containing a selected transgene under the control of sequences which direct expression thereof and AAV-1 capsid proteins.

Optionally, a sample from the host may be first assayed for the presence of antibodies to a selected AAV serotype. A variety of assay formats for detecting neutralizing antibodies are well known to those of skill in the art. The selection of such an assay is not a limitation of the present invention. See, e.g., Fisher et al, *Nature Med.*, 3(3):306–312 (March 1997) and W. C. Manning et al, *Human Gene Therapy*, 9:477–485 (Mar. 1, 1998). The results of this assay may be used to determine which AAV vector containing capsid proteins of a particular serotype are preferred for delivery, e.g., by the absence of neutralizing antibodies specific for that capsid serotype.

In one aspect of this method, the delivery of vector with AAV-1 capsid proteins may precede or follow delivery of a gene via a vector with a different serotype AAV capsid protein. Thus, gene delivery via rAAV vectors may be used for repeat gene delivery to a selected host cell. Desirably, subsequently administered rAAV vectors carry the same transgene as the first rAAV vector, but the subsequently administered vectors contain capsid proteins of serotypes which differ from the first vector. For example, if a first vector has AAV-2 capsid proteins, subsequently administered vectors may have capsid proteins selected from among the other serotypes, including AAV-1, AAV-3A, AAV-3B, AAV-4 and AAV-6.

Thus, a rAAV-1-derived recombinant viral vector of the invention provides an efficient gene transfer vehicle which can deliver a selected transgene to a selected host cell in vivo or ex vivo even where the organism has neutralizing antibodies to one or more AAV serotypes. These compositions are particularly well suited to gene delivery for therapeutic purposes. However, the compositions of the invention may also be useful in immunization. Further, the compositions of the invention may also be used for production of a desired gene product in vitro.

The above-described recombinant vectors may be delivered to host cells according to published methods. An AAV viral vector bearing the selected transgene may be administered to a patient, preferably suspended in a biologically compatible solution or pharmaceutically acceptable delivery vehicle. A suitable vehicle includes sterile saline. Other aqueous and non-aqueous isotonic sterile injection solutions and aqueous and non-aqueous sterile suspensions known to be pharmaceutically acceptable carriers and well known to those of skill in the art may be employed for this purpose.

The viral vectors are administered in sufficient amounts to transfect the cells and to provide sufficient levels of gene transfer and expression to provide a therapeutic benefit without undue adverse effects, or with medically acceptable physiological effects, which can be determined by those skilled in the medical arts. Conventional and pharmaceutically acceptable routes of administration include, but are not limited to, direct delivery to the liver, oral, intranasal, intravenous, intramuscular. subcutaneous, intradermal, and other parental routes of administration. Routes of administration may be combined, if desired.

Dosages of the viral vector will depend primarily on factors such as the condition being treated, the age, weight and health of the patient, and may thus vary among patients. For example, a therapeutically effective human dosage of the viral vector is generally in the range of from about 1 ml to about 100 ml of solution containing concentrations of from about 1×10^9 to 1×10^{16} genomes virus vector. A preferred human dosage may be about 1×10^{13} to 1×10^{16} AAV genomes. The dosage will be adjusted to balance the therapeutic benefit against any side effects and such dosages may

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Similarly, one of skill in the art can readily select other fragments of the AAV-1 genome of the invention for use in a variety of vector systems. Such vectors systems may include, e.g., lentiviruses, retroviruses, poxviruses, vaccinia viruses, and adenoviral systems, among others. Selection of these vector systems is not a limitation of the present invention.

C. Host Cells And Packaging Cell Lines

In yet another aspect, the present invention provides host cells which may be transiently transfected with AAV-1 nucleic acid sequences of the invention to permit expression of a desired transgene or production of a rAAV particle. For example, a selected host cell may be transfected with the AAV-1 P5 promoter sequences and/or the AAV-1 5' ITR sequences using conventional techniques. Providing AAV helper functions to the transfected cell lines of the invention results in packaging of the rAAV as infectious rAAV particles. Such cell lines may be produced in accordance with known techniques [see, e.g, US Patent No. 5,658,785], making use of the AAV-1 sequences of the invention.

Alternatively, host cells of the invention may be stably transfected with a rAAV expression cassette of the invention, and with copies of AAV-1 rep and cap genes. Suitable parental cell lines include mammalian cell lines and it may be desirable to select host cells from among non-simian mammalian cells. Examples of suitable parental cell lines include, without limitation, HeLa [ATCC CCL 2], A549 [ATCC Accession No. CCL 185], KB [CCL 17], Detroit [e.g., Detroit 510, CCL 72] and WI-38 [CCL 75] cells. These cell lines are all available from the American Type Culture Collection, 10801 University Boulevard, Manassas, Virginia 20110-2209 USA. Other suitable parent cell lines may be obtained from other sources and may be used to construct stable cell lines containing the P5 and/or AAV rep and cap sequences of the invention.

Recombinant vectors generated as described above are useful for delivery of the DNA of interest to cells.

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NAB titers were analyzed by assessing the ability of serum antibody to inhibit the transduction of reporter virus expressing green fluorescent protein (GFP) (AAV1-GFP or AAV2-GFP) into 84-31 cells. Various dilutions of antibodies preincubated with reporter virus for 1 hour at 37° C. were added to 90% confluent cell cultures. Cells were incubated for 48 hours and the expression of green fluorescent protein was measured by FluoroImaging (Molecular Dynamics). NAB titers were calculated as the highest dilution at which 50% of the cells stained green.

Analysis of NAB in rhesus monkeys showed that 61% of animals tested positive for AAV-1; a minority (24%) has NAB to AAV-2. Over one-third of animals had antibodies to AAV-1 but not AAV-2 (i.e., were monospecific for AAV-1), whereas no animals were positive for AAV-2 without reacting to AAV-1. These data support the hypothesis that AAV-1 is endemic in rhesus monkeys. The presence of true AAV-2 infections in this group of nonhuman primates is less clear, since cross-neutralizing activity of an AAV-1 response to AAV-2 can not be ruled out. It is interesting that there is a linear relationship between AAV-2 NAB and AAV-1 NAB in animals that had both.

B. Humans

For these neutralization antibody assays, human serum samples were incubated at 56° C. for 30 min to inactivate complement and then diluted in DMEM. The virus (rAAV or rAd with either lacZ or GFP) was then mixed with each serum dilution (20×, 400×, 2000×, 4000×, etc.) and incubated for 1 hour at 37° C. before applied to 90% confluent cultures of 84-31 cells (for AAV) or Hela cells (for adenovirus) in 96-well plates. After 60 minutes of incubation at culture condition, 100 μl additional media containing 20% FCS was added to make final culture media containing 10% FCS.

The result is summarized in Table 3.

TABLE 3

Adenovirus	AAV-1	AAV-2	# of samples	Percentage
−	−	−	41	53.2%
+	−	−	16	20.8%
−	+	−	0	0.0%
−	−	+	2	2.6%
−	+	+	2	2.6%
+	−	+	3	3.9%
+	+	−	0	0.0%
+	+	+	13	16.9%
		Total	77	100%

The human neutralizing antibodies against these three viruses seemed to be unrelated since the existence of neutralizing antibodies against AAV are not indications for antibodies against adenovirus. However, AAV requires adenovirus as helper virus, in most of the cases, the neutralizing antibodies against AAV correlated with the existence of neutralizing antibodies to adenovirus. Among the 77 human serum samples screened, 41% of the samples can neutralize the infectivity of recombinant adenovirus based on Ad5. 15/77 (19%) of serum samples can neutralize the transduction of rAAV-1 while 20/77 (20%) of the samples inhibit rAAV-2 transduction at 1 to 80 dilutions or higher. All serum samples positive in neutralizing antibodies for AAV-1 in are also positive for AAV-2. However, there are five (6%) rAAV-2 positive samples that failed to neutralize rAAV-1. In samples that are positive for neutralizing antibodies, the titer of antibodies also varied in the positive ones. The results from screening human sera for antibodies against AAVs supported the conclusion that AAV-1 presents the same epitome as that of AAV-2 to interact with cellular receptors since AAV-1 neutralizing human serums can also decrease the infectivity of AAV-2. However, the profile of neutralizing antibodies for these AAVs is not identical, there are additional specific receptors for each AAV serotype.

EXAMPLE 6

Recombinant AAV Viruses Exhibit Tissue Tropism

The recombinant AAV-1 vectors of the invention and the recombinant AAV-2 vectors [containing the gene encoding human α1-antitrypsin (α1AT) or murine erythropoietin (Epo) from a cytomegalovirus-enhanced β-actin promoter (CB)] were evaluated in a direct comparison to equivalent copies of AAV-2 vectors containing the same vector genes.

Recombinant viruses with AAV-1 capsids were constructed using the techniques in Example 1. To make rAAV with AAV-1 virions, pAV1H or p5E18 (2/1) was used as the trans plasmid to provide Rep and Cap functions. For the generation of the rAAV based on AAV-2, p5E18(2/2) was used as the trans plasmid, since it greatly improved the rAAV yield. [Early experiments indicated similar in vivo performances of AAV-1 vectors produced with pAV1H and p5E19 (2/1). All subsequent studies used AAV-1 vectors derived from p5E18(2/1) because of the increased yield.]

Equivalent stocks of the AAV-1 and AAV-2 vectors were injected intramuscularly (5×10^{1} genomes) or liver via the portal circulation (1×10^{11} genomes) into immunodeficient mice, and the animals (four groups) were analyzed on day 30 for expression of transgene. See, FIGS. 4A and 4B.

AAV-2 vectors consistently produced 10- to 50-fold more serum erythropoietin or α1-antitrypsin when injected into liver compared to muscle. (However, the AAV-1-delivered genes did achieve acceptable expression levels in the liver.) This result was very different from that for AAV-1 vectors, with which muscle expression was equivalent to or greater than liver expression. In fact, AAV-1 outperformed AAV-2 in muscle when equivalent titers based on genomes were administered.

EXAMPLE 7

Gene Delivery via rAAV-1

C57BL/6 mice (6- to 8-week old males, Jackson Laboratories) were analyzed for AAV mediated gene transfer to liver following intrasplenic injection of vector (i.e., targeted to liver). A total of 10^{11} genome equivalents of rAAV-1 or rAAV-2 vector were injected into the circulation in 100 μl buffered saline. The first vector contained either an AAV-1 capsid or an AAV-2 capsid and expressed α1AT under the control of the chicken β-actin (CB) promoter. Day 28 sera were analyzed for antibodies against AAV-1 or AAV-2 and serum α1AT levels were checked. Animals were then injected with an AAV-1 or AAV-2 construct expressing erythropoietin (Epo, also under the control of the CB promoter). One month later sera was analyzed for serum levels of Epo. The following groups were analyzed (FIGS. 5A–5D).

In Group 1, vector 1 was AAV-2 expressing α1AT and vector 2 was AAV-2 expressing Epo. Animals generated antibodies against AAV-2 following the first vector administration which prevented the readministration of the AAV-2 based vector. There was no evidence for cross-neutralizing the antibody to AAV-1.

In Group 2, vector 1 was AAV-1 expressing α1AT while vector 2 was AAV-1 expressing Epo. The first vector admin-

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with cellular receptors since AAV-1 neutralizing human serums can also decrease the infectivity of AAV-2. However, the profile of neutralizing antibodies for these AAVs is not identical, there are additional specific receptors for each AAV serotype.

Example 6 - Recombinant AAV Viruses Exhibit Tissue Tropism

The recombinant AAV-1 vectors of the invention and the recombinant AAV-2 vectors [containing the gene encoding human α1-antitrypsin (α1AT) or murine erythropoietin (Epo) from a cytomegalovirus-enhanced β-actin promoter (CB)] were evaluated in a direct comparison to equivalent copies of AAV-2 vectors containing the same vector genes.

Recombinant viruses with AAV-1 capsids were constructed using the techniques in Example 1. To make rAAV with AAV-1 virions, pAV1H or p5E18 (2/1) was used as the *trans* plasmid to provide Rep and Cap functions. For the generation of the rAAV based on AAV-2, p5E18(2/2) was used as the *trans* plasmid, since it greatly improved the rAAV yield. [Early experiments indicated similar *in vivo* performances of AAV-1 vectors produced with pAV1H and p5E19 (2/1). All subsequent studies used AAV-1 vectors derived from p5E18(2/1) because of the increased yield.]

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-continued

aggagagcgc cggagcttca aacactgcat tggacaatgt catgatcaca gacgaagagg 3900

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caa 4683

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<211> LENGTH: 16
<212> TYPE: DNA
<213> ORGANISM: rep binding motif

<400> SEQUENCE: 20

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What is claimed is:

1. A recombinant virus having an AAV-1 capsid comprising an AAV-1 protein selected from among AAV-1 vp1 having the amino acid sequence of SEQ ID NO:13; AAV-1 vp2 having the amino acid sequence of SEQ ID NO:15and AAV-1 vp3 having the amino acid sequence of SEQ ID NO:17 and a heterologous molecule which comprises an AAV 5' inverted terminal repeat sequence (ITR), a transgene, and an AAV 3' ITR.

2. The recombinant virus according to claim 1, wherein the 5' ITR and 3' ITR arc of AAV serotype 2.

3. The recombinant virus according to claim 1 further comprising a regulatable promoter which directs expression of the transgene.

4. A recombinant host cell transformed with the recombinant virus of claim 1.

5. The recombinant virus according to claim 1, wherein said virus comprises an AAV-1 capsid comprising the AAV-1 vp1 having the amino acid sequence of SEQ ID NO:13.

6. The recombinant virus according to claim 1, wherein said virus comprises an AAV-1 capsid comprising the AAV-1 vp2 having the amino acid sequence of SEQ ID NO:15.

7. The recombinant virus according to claim 1, wherein said virus comprises an AAV-1 capsid comprising the AAV-1 vp3 having the amino acid sequence of SEQ ID NO:17.

8. The recombinant virus according to claim 1, wherein the 5' ITR and 3' ITR are of AAV serotype 1.

9. A composition comprising a carrier and the recombinant virus according to claim 1.

* * * * *

See page 2 of preliminary Amendment. (Attached)

ERROR (9) (See page 2)

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the Application of) Group Art Unit: 1635
)
James M. Wilson et al) Examiner: B. Whiteman
)
Appln. No. 09/807,802)
)
Filed: November 29, 2001)
)
For: ADENO-ASSOCIATED VIRUS)
SEROTYPE I NUCLEIC ACID)
SEQUENCES, VECTORS AND HOST)
CELLS CONTAINING SAME)

Assistant Commissioner for Patents
Washington, DC 20231

THIRD PRELIMINARY AMENDMENT

Sir:

Please amend the application as set forth below.

In the Claims

Kindly add new claims 33 - 40.

CERTIFICATE OF TRANSMISSION under 37 CFR 1.8
I hereby certify that this correspondence is being facsimile transmitted to the United States Patent and Trademark Office on December 31, 2002.

Cathy A Kodroff
Signature

Cathy A. Kodroff
Typed or printed name of person signing Certificate

33. A recombinant virus having an AAV-1 capsid comprising an AAV-1 protein selected from among AAV-1 vp1 having the amino acid sequence of SEQ ID NO:13; AAV-1 vp2 having the amino acid sequence of SEQ ID NO:15 and AAV-1 vp3 having the amino acid sequence of SEQ ID NO:17 and a heterologous molecule which comprises an AAV 5' inverted terminal repeat sequence (ITR), a transgene, and an AAV 3' ITR.

34. The recombinant virus according to claim 33, wherein the 5' ITR and 3' ITR are of AAV serotype 2.

35. The recombinant virus according to claim 33 further comprising a regulatable promoter which directs expression of the transgene.

36. A recombinant host cell transformed with the recombinant virus of claim 33.

37. A recombinant host cell transformed with a nucleic acid sequence expressing one or more AAV-1 rep proteins selected from among rep78 having the amino acid sequence of SEQ ID NO:5, rep 68 having the amino acid sequence of SEQ ID NO:7, rep 52 having the amino acid sequence of SEQ ID NO: 9, and rep 40 having the amino acid sequence of SEQ ID NO:11.

38. A recombinant host cell transformed with a nucleic acid sequence expressing one or more AAV-1 cap proteins selected from among vp1 having the amino acid sequence of SEQ ID NO:13, vp2 having the amino acid sequence of SEQ ID NO: 15 and vp3 having the amino acid sequence of SEQ ID NO:17.

39. A method for transducing a muscle cell, said method comprising the step of infecting the cell with a recombinant AAV vector comprising an AAV1 capsid.

40. A method for transducing a liver cell, said method comprising the step of infecting the cell with a recombinant AAV vector comprising an AAV1 capsid.

REMARKS

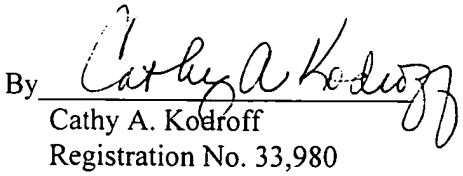
After entry of this preliminary amendment, the pending claims are claims 1-6, 9-11, 14, 16-20, and 23 - 40.

New claims 33 - 40 are supported on page 9, lines 22; page 11, lines 7-11; page 14, lines 6-8 and 16-18; page 24, lines 18-20; Example 4, page 26, lines 2 - 11 and Example 6, page 29, lines 25 - 28, and throughout the specification. No new matter is introduced by this preliminary amendment.

Attached hereto is a clean copy of the claims as amended by this paper.

Applicants hereby authorize the additional claim fees to be charged to Deposit Account No. 08-3040. Additionally, the Director of the U. S. Patent and Trademark Office is hereby authorized to charge any other fees due with the filing of this paper to Deposit Account No. 08-3040.

Respectfully submitted,
HOWSON AND HOWSON
Attorneys for Applicants

By 

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TO: Group Art Unit 1635

DATE: December 31, 2002

REFERENCE: US Patent Application No. 09/807,802

NUMBER OF PAGES (INCLUDING COVER SHEET): 12

FAX NUMBER OF RECIPIENT: (703) 308-4242

Dear Sirs:

OFFICIAL

Enclosed is a twelve page submission, composed of a Third Preliminary Amendment (4 pages), a clean copy of the pending claims (6 pages); a fee transmittal form (1 page); and this transmittal cover sheet (1 page)

Respectfully submitted,
HOWSON AND HOWSON

By: Cathy A. Kodroff
Cathy A. Kodroff
Registration No. 33,980

CERTIFICATE OF TRANSMISSION under 37 CFR 1.8

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Cathy A. Kodroff
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Typed or printed name of person signing Certificate

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PTO/SB/17 (10-01)
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FEE TRANSMITTAL for FY 2002

Patent fees are subject to annual revision.

TOTAL AMOUNT OF PAYMENT ($) 564.00

Complete if Known	
Application Number	09/807,802
Filing Date	11/29/2001
First Named Inventor	James M. Wilson et al
Examiner Name	B. Whiteman
Group Art Unit	1635
Attorney Docket No.	GNVPN.031US

METHOD OF PAYMENT

1. ☑ The Commissioner is hereby authorized to charge indicated fees and credit any overpayments to:
   Deposit Account Number: 08-3040
   Deposit Account Name: HOWSON AND HOWSON
   ☑ Charge Any Additional Fee Required Under 37 CFR 1.16 and 1.17
   ☐ Applicant claims small entity status. See 37 CFR 1.27
2. ☐ **Payment Enclosed:**
   ☐ Check ☐ Credit card ☐ Money Order ☐ Other

FEE CALCULATION

1. BASIC FILING FEE

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
101	740	201	370	Utility filing fee	
106	330	206	165	Design filing fee	
107	510	207	255	Plant filing fee	
108	740	208	370	Reissue filing fee	
114	160	214	80	Provisional filing fee	

SUBTOTAL (1) ($)

2. EXTRA CLAIM FEES

			Extra Claims		Fee from below	Fee Paid
Total Claims	33	-20** =	8	x	18.00	= 144.00
Independent Claims	15	-3** =	5	x	84.00	= 420.00
Multiple Dependent						=

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description
103	18	203	9	Claims in excess of 20
102	84	202	42	Independent claims in excess of 3
104	280	204	140	Multiple dependent claim, if not paid
109	84	209	42	** Reissue independent claims over original patent
110	18	210	9	** Reissue claims in excess of 20 and over original patent

SUBTOTAL (2) ($) 564.00

**or number previously paid, if greater; For Reissues, see above

FEE CALCULATION (continued)

3. ADDITIONAL FEES

Large Entity Fee Code	Large Entity Fee ($)	Small Entity Fee Code	Small Entity Fee ($)	Fee Description	Fee Paid
105	130	205	65	Surcharge - late filing fee or oath	
127	50	227	25	Surcharge - late provisional filing fee or cover sheet	
139	130	139	130	Non-English specification	
147	2,520	147	2,520	For filing a request for *ex parte* reexamination	
112	920*	112	920*	Requesting publication of SIR prior to Examiner action	
113	1,840*	113	1,840*	Requesting publication of SIR after Examiner action	
115	110	215	55	Extension for reply within first month	
116	400	216	200	Extension for reply within second month	
117	920	217	460	Extension for reply within third month	
118	1,440	218	720	Extension for reply within fourth month	
128	1,960	228	980	Extension for reply within fifth month	
119	320	219	160	Notice of Appeal	
120	320	220	160	Filing a brief in support of an appeal	
121	280	221	140	Request for oral hearing	
138	1,510	138	1,510	Petition to institute a public use proceeding	
140	110	240	55	Petition to revive - unavoidable	
141	1,280	241	640	Petition to revive - unintentional	
142	1,280	242	640	Utility issue fee (or reissue)	
143	460	243	230	Design issue fee	
144	620	244	310	Plant issue fee	
122	130	122	130	Petitions to the Commissioner	
123	50	123	50	Processing fee under 37 CFR 1.17(q)	
126	180	126	180	Submission of Information Disclosure Stmt	
581	40	581	40	Recording each patent assignment per property (times number of properties)	
146	740	246	370	Filing a submission after final rejection (37 CFR § 1.129(a))	
149	740	249	370	For each additional invention to be examined (37 CFR § 1.129(b))	
179	740	279	370	Request for Continued Examination (RCE)	
169	900	169	900	Request for expedited examination of a design application	

Other fee (specify) _____

*Reduced by Basic Filing Fee Paid SUBTOTAL (3) ($)

SUBMITTED BY				Complete (if applicable)	
Name (Print/Type)	Cathy A. kodroff	Registration No. (Attorney/Agent)	33,980	Telephone	215-540-9200
Signature	Cathy A. Kodroff			Date	12/31/2002

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